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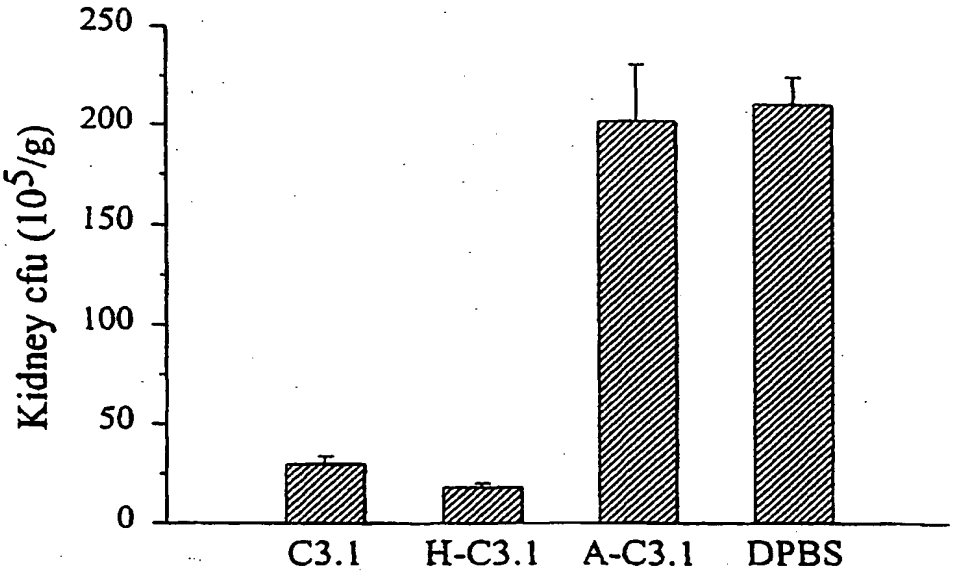

(57) Abstract

Antibodies are disclosed that protect against candidiasis, particularly antibodies of the IgG class and antibodies that specifically bind to a carbohydrate antigen of the cell wall of a yeast from the *Candida* genus. Also disclosed are pharmaceutical compositions and therapeutic methods useful in the treatment of candidiasis as well as diagnostic methods.

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ANTIBODIES AGAINST PHOSPHOMANNAN THAT ARE PROTECTIVE AGAINST CANDIDIASIS

FIELD OF THE INVENTION

The present invention relates to antibodies of the IgG class that protect a host against candidiasis, particularly to antibodies that specifically bind to a carbohydrate antigen of the cell wall of a yeast from the *Candida* genus. The invention further relates to pharmaceutical compositions and therapeutic methods useful in the treatment of candidiasis and diagnostic methods useful in diagnosing candidiasis and monitoring the course of treatment of candidiasis.

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BACKGROUND OF THE INVENTION

The yeast genera, *Candida,* can cause a variety of clinical syndromes that are generically termed candidiasis and are usually categorized by the physiological site of involvement. The two most common syndromes are mucocutaneous candidiasis (*e.g.,* stomatitis or thrush, esophagitis and vaginitis) and invasive or deep organ candidiasis (*e.g.*, fungemia, endocarditis, and endophthalmitis). These syndromes are discussed in Dismukes, *Candidiasis*, IN CECIL'S TEXTBOOK OF MEDICINE 1827-1830 (Bennett *et al.* eds., 1996).

Patients suffering from mucocutaneous infections may be treated with any one of several topical preparations including nystatin, clotrimazole, econazole, ketoconazole, butoconazole, terconazole, and miconazole. *Id.* For the treatment of more clinically serious *Candida* related disease (*e.g.*, candidemia or disseminated candidiasis)

amphotericin B formulations, both the deoxycholate version and the newer liposomal product, are administered only intravenously. Fluconazole is oral. Therapy for *Candida* peritonitis involves either intravenous amphotericin B or oral gluconazole. *Id.*

The medical literature also reported that various classes of antibodies (IgA, IgG and IgM) directed against several *C. albicans* antigens were of experimental interest and of diagnostic or therapeutic value. See, *e.g.*, Torres-Rodriguez *et al.*, 1997 *Mycoses* 40:439-44 with respect to *C. albicans* germ tube; and Reboli, 1993 *J. Clin. Microbiol.* 31: 518-23 with respect to a dot immunobinding assay involving total *Candida* protein. Monoclonal antibodies specific to an iC3b receptor, which is an integrin that has antigenic and structural homology with a *Candida* surface antigen, were demonstrated to increase survival of mice with disseminated candidiasis. Lee *et al.*, 1997 *Immunology* 92: 104-110. Similarly, antibodies to mannoprotein (MP) and aspartyl proteinase (Sap) have been shown to protect against vaginitis in rats. De Bernardis *et al.*, 1997 *Infect. Immun.* 65: 3399-3405.

U.S. Patents No. 4,670,382 and 4,806,465 to Buckley *et al.* (1989) describe IgG monoclonal antibodies against a set of closely related cytoplasmic antigens of *C. albicans,* but present no therapeutic data showing efficacy against *Candida* infection. U.S. Patent No. 5,288,639 to Burnie *et al.* (1994) describes monoclonal antibodies against stress or heat shock proteins of *Candida,* which were shown to produce 33 % survival at 24 hours in animals challenged with a lethal dose of *C. albicans*. Also, U.S. Patent No. 5,641,760 to Yu *et al.* (1997) discloses monoclonal antibodies against *C. albicans* fimbrial subunits that are said to be useful for treating *C. albicans* infections. However, although this patent identifies antibodies as members of the IgG2 isotype, no *in vivo* data showing protection against *Candida* infection were provided.

Certain immunogenic phosphomannan preparations of *C. albicans,* which is known to contain adhesins, have been used to prepare vaccines for the treatment of, and elicit antibodies against, disseminated candidiasis due to infection by *C. albicans*. For example, European Patent No. 344,320 to Kawamura *et al.* (1989) describes human monoclonal antibodies of IgG and IgM classes that were raised against mannan extracted from *Candida*. Although antibodies of the IgG class are said to be preferred and

agglutinating activity is discussed, the skilled artisan will understand that agglutination is distinct from protective effect and no therapeutic data against *Candida* infection were provided by Kawamura *et al.*.

Therapeutic efficacy was shown in U.S. Patent No. 5,578,309 to Cutler *et al.* (1996) described the immunization of mice with liposome-encapsulated *Candida* phosphomannoprotein and obtained several monoclonal antibodies specific for that fraction. In addition, mice were immunized with a liposome encapsulated mannan adhesin extracted from the cell wall ("L-adhesin" or "L-mannan" or "L-mann"), and two IgM class monoclonal antibodies specific for yeast surface epitopes were described in Cutler *et al.* Although both antibodies (B6.1 and B6) were strong agglutinins, only one (B6.1) was shown to protect naive mice against disseminated candidiasis. Each antibody recognizes a distinct *C. albicans* mannan cell wall determinant, and the MAb B6.1 recognized a carbohydrate antigen. See, also, Han *et al.*, 1997 *Infect. Immun.* 65: 4100-07. The B6.1 antibody also enhanced ingestion and killing of yeast cells by polymorphonuclear leukocytes (PMNs) in the presence of serum complement. Caesar-TonThat *et al.*, 1997 *Infect. Immun.* 65: 5354-57.

Thus, unlike the disclosure of the B6.1 antibody, which is an IgM class antibody, the other literature citations discussed above apparently did not demonstrate protection against candidiasis by antibodies of the IgG class that were specific for the phosphomannan antigen of the *Candida* cell wall. Notwithstanding the work by Cutler *et al.*, it was surprising that a protective IgG class antibody has been found by the present inventors that is specific for a carbohydrate antigen.

With respect to antibody class, class switching does not guarantee similar levels of effectiveness. Thus, the reported effectiveness of IgM antibodies such as the B6.1 antibody does not mean that antibodies of a different class even when directed against the same antigen will be similarly effective in a clinical setting. For example, in Group B streptococcal disease, an IgM specific for a cell wall carbohydrate was much more effective than an IgG2a of the same specificity as the IgM. Hill *et al.*, 1992 *Clin. Immunol. Immunopathol.* 62: 87-91. Similarly, isotype switching can convert protective antibodies to nonprotective antibodies. For example, with respect to *Cryptococcus*

neoformans, isotype switching from IgG3 to IgG1, while maintaining identical epitope specificity, converted a nonprotective antibody to a protective one in experimental cryptococcosis. Yuan *et al.*, 1995 *J. Immunol.* 154: 1810-1816; and Yuan *et al.*, 1998 *Infect. Immun.* 66: 1057-1062.

As is known in the art, antibodies of the IgM class can fix complement and directly activate macrophages. Because IgG class antibodies can activate complement as well as bind to macrophages and neutrophils, they are able to enhance immunity by antibody mediated and cellular responses. IgG antibodies also may have higher binding affinities than do IgM antibodies. The immune responses mediated by IgM may have quite different outcomes in terms of how a mammalian host infected with *Candida* is able to recognize and respond to this pathogen, because both antibody and cellular responses are known to be important in responding to *Candida* infection. Thus, the use of both IgG and IgM antibodies, administered together or perhaps sequentially, may help to boost an infected mammalian host's immune response. Although the B6.1 IgM antibody is known to recognize a phosphomannan adhesin and is protective, no monoclonal IgG antibodies, until the invention described herein, had been described in the literature that recognized a *Candida* adhesin and were therapeutically effective in inhibiting or preventing candidiasis.

SUMMARY OF THE INVENTION

The present invention relates to antibodies that protect a host against candidiasis, particularly disseminated candidiasis, mucocutaneous candidiasis (*e.g.*, stomatitis or thrush, esophagitis and vaginitis or vaginal candidiasis) and invasive or deep organ candidiasis (*e.g.*, fungemia, endocarditis, and endophthalmitis). More particularly, the invention relates to antibodies of the IgG class and IgG3 isotype. The invention also relates to therapeutic methods useful in the treatment of candidiasis and diagnostic methods useful in diagnosing candidiasis and monitoring the course of treatment of candidiasis.

It is an object of the present invention to provide a purified or isolated IgG antibody that specifically binds to a carbohydrate antigen of the cell wall of a yeast from

the *Candida* genus, where the antibody is protective against infection of a mammalian host by the yeast. More particularly, it is an object of the invention to provide an antibody that specifically binds to an acid-labile component of the phosphomannan complex, particularly a β-1,2-linked oligomannosyl or a β-1,2-mannotriose residue, of the cell wall of the yeast.

The foregoing antibodies are protective against a several types of candidiasis, including disseminated candidiasis and mucocutaneous candidiasis. The antibody may be of any IgG isotype, including IgG1, IgG2 and IgG3 and their various sub-isotypes. Also contemplated are human antibodies, chimeric antibodies or humanized antibodies.

It is a further object of the invention to provide pharmaceutical compositions comprising the foregoing antibodies, formulated with pharmaceutically acceptable carriers and excipients as appropriate. Contemplated yeast infections treatable by such pharmaceutical compositions include *C. albicans, C. glabrata* and *C. tropicalis* and strains thereof. Topical, systemic and aerosol formulations are expressly contemplated, as are formulations in unit dose form and in formulations containing one or more other anti-fungal, antibody or other therapeutic agents. In a preferred formulation, both IgG and IgM antibodies are administered against the same yeast, either at about the same time or at different times.

Another object of the invention is to utilize the foregoing pharmaceutical compositions in methods to treat disseminated candidiasis and mucocutaneous candidiasis. Diagnostic kits comprising the antibodies described above, together with a reagent for detecting binding of the antibody to a carbohydrate antigen of the cell wall of a yeast from the *Candida* genus also are contemplated, as are hybridoma cells that express these antibodies.

BRIEF DESCRIPTION OF THE DRAWING FIGURES

Figs. 1A and 1B show that passive transfer of MAb C3.1 protects against disseminated candidiasis in BALB/c mice.

Figs. 2A and 2B show that MAb C3.1 has a prophylactic effect against *Candida* vaginal infection in pseudoestrus mice.

DETAILED DESCRIPTION OF THE INVENTION

1. *Definitions*

The term "antibody" as used herein, unless indicated otherwise, is used broadly to refer to both antibody molecules and a variety of antibody-derived molecules. Such antibody-derived molecules comprise at least one variable region (either a heavy chain or light chain variable region) and include molecules such as Fab fragments, Fab' fragments, $F(ab')_2$ fragments, Fv fragments, Fabc fragments, single chain Fv (scFv) antibodies, individual antibody light chains, individual antibody heavy chains, chimeric fusions between antibody chains and other molecules, and the like.

Antibodies of the invention may be isolated from a hybridoma cell, the serum of a vertebrate, recombinant eukaryotic or prokaryotic cells transfected with a nucleic acid encoding the antibody, which may include plant cells, ascites fluid, or the milk of transgenic animals.

The term "antigen" means a molecule that is specifically recognized and bound by an antibody. The specific portion of the antigen that is bound by the antibody is termed the "epitope".

The term "humanized antibody" refers to an antibody which is substantially human in structure; that is, it derives at least substantially all of its constant regions from a human antibody even though all or a part of its variable regions are derived from some other species. "Human antibody" refers to an antibody which is encoded by a nucleotide of human origin and such nucleotides may be modified by the skilled artisan by known nucleotide manipulation techniques.

Antibodies described herein also may contain alterations of the amino acid sequence compared to a naturally occurring antibody. In other words, the antibodies of the invention need not necessarily consist of the precise amino acid sequence of their native variable region or constant region framework, but contain various substitutions that improve the binding properties of the antibody to its cognate antigen or change the binding of the antibody to effector molecules such as complement or the Fc receptor. In another format, a minimal number of substitutions are made to the framework region in order to ensure reduced, and preferably, minimal immunogenicity of the antibody in

humans. In preferred embodiments of recombinant antibodies of the invention, any non-human framework regions used may be altered with a minimal number of substitutions to the framework region in order to avoid large-scale introductions of non-human framework residues.

The term "conventional molecular biology methods" refers to techniques for manipulating polynucleotides that are well known to the person of ordinary skill in the art of molecular biology. Examples of such well known techniques can be found in MOLECULAR CLONING: A LABORATORY MANUAL 2ND EDITION, Sambrook *et al.*, Cold Spring Harbor, N.Y. (1989) and in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausebel *et al.*, 4 vols. John Wiley & Sons, NY. Examples of conventional molecular biology techniques include, but are not limited to, *in vitro* ligation, restriction endonuclease digestion, polymerase chain reaction (PCR), cellular transformation, hybridization, electrophoresis, DNA sequencing, cell culture, and the like.

The term "isolated" or "substantially pure" as used herein refers to an antibody or, for example, a fragment thereof, which is substantially free of other antibodies, proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art would be able to isolate or to substantially purify MAb C3.1 antibodies using conventional methods for antibody or protein purification

The terms "protective" or "therapeutically effective" generally mean that the antibody is effective to block attachment of a yeast cell to its target tissue or cells in a host, or to decrease or prevent the increase in fungal cell levels in the bloodstream or at an organ site or other site of infection. More specifically, the phrase "protective" or "therapeutically effective" means that the antibodies or pharmaceutical compositions according to the present invention are able to opsonize *Candida* pathogens to facilitate macrophage, monocyte or neutrophil phagocytosis and killing, or can activate the macrophages that can amplify the cellular and immune responses. Preferably, the treatment methods of the present invention are effective to kill at least about 20 %, more preferably 40%, even more preferably 60% and most preferably 90% or more of the *Candida* organisms in an infected mammalian host in a therapeutic course of treatment.

The terms "variable region" and "constant region" as used herein in reference to antibody and immunoglobulin molecules have the ordinary meaning given to the term by a person of ordinary skill in the art of immunology. Both antibody heavy chains and antibody light chains may be divided into a "variable region" and a "constant region." The point of division between a variable region and a contrast region may be determined by the person of ordinary skill in the art by reference to standard texts describing antibody structure. See, *e.g.*, Kabat *et al.*, "Sequences of Proteins of Immunological Interest: 5th Edition" U.S. Department of Health and Human Services, U.S. Government Printing Office (1991).

2. *Candida Related Conditions*

Among the more than 150 recognized species of *Candida, C. albicans* is the most commonly identified pathogen in humans. Other clinically important species include *C. guilliermondi, C. krusei, C. parapsilosis, C. pseudotropicalis* and *C. tropicalis*. Mucocutaneous infections include thrush or oropharyngeal candidiasis, cheilosis, esophagitis, gastrointestinal candidiasis, intertrigo, paronychia, vulvovaginitis, balanitis, *Candida* cystitis, and chronic mucocutaneous candidiasis. Numerous diagnostic categories exist for serious or deep *Candida* infection including candidemia, disseminated candidiasis, systemic candidiasis, invasive candidiasis, visceral candidiasis and terms indicating involvement of specific organs such as hepatosplenic candidiasis and ocular candidiasis. See, *e.g.*, Dismukes, 1996. Serious or deep *Candida* infections are frequently observed in immunodeficient or immune compromised patients, such as in patients with Acquired Immundeficiency Syndrome (AIDS).

3. *Preparation of Hybridomas that Produce Monoclonal Antibodies Against Candida antigens*.

To produce antibodies, various species of host animals may be immunized by injection with the L-mann antigen or with appropriately prepared *Candida* extracts or whole cells. Appropriate animals for this purpose include, but are not limited to rabbits, mice, and rats, etc. Various adjuvants may be used to increase the immunological

response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacilli Calmette-Guerin) and Corynebacterium parvum.

Monoclonal antibodies to *Candida* antigens may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein *(Nature*, 1975, 256:495-497), the human B-cell hybridoma technique (Kosbor *et al.*, 1983, *Immunology Today*, 4:72; Cote *et al.*, 1983, *Proc. Natl. Acad. Sci.*, 80:2026-2030) and the EBV- hybridoma technique (Cole *et al.*, 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In addition, techniques developed for the production of "chimeric antibodies" (Morrison *et al.*, 1984, *Proc. Natl. Acad. Sci.*, 81:6851-6855; Neuberger *et al.*, 1984, *Nature*, 312:604-608; Takeda *et al.*, 1985, *Nature*, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce *Candida* specific single chain antibodies.

4. *Isolation of Candida antigen specific B cells.*

Antigen specific B cells may be isolated from convenient samples, such as peripheral blood lymphocytes from a human patient infected with *Candida*, by techniques known and available in the art. For instance, fusion proteins of the invention may be used to detect and isolate B cells which express immunoglobulin which specifically binds to the phosphomannan antigen by affinity chromatography, fluorescent activated cell sorting (FACS) and other commonly used techniques such as Zn-chelating sepharose or protein-A sepharose (see Harlow *et al.*, ANTIBODIES: A LABORATORY MANUAL, Cold Spring Harbor Laboratory, 1988).

As another example, lymph nodes obtained from a candidiasis patient may be cut into fine pieces and meshed through a wire gauze using a rubber policeman. Pure B cells may be isolated using CD19 coated immunomagnetic beads. Antigen specific B cells may be isolated using the appropriate fusion protein by affinity chromatography or fluorescent activated cell sorting. The *Candida* antigen specific B cells may then be immortalized using known techniques such as immortalization by EBV. Any effective lymphotropic virus or other transforming agent able to transform the B-cells to grow in continuous culture and still produce monoclonal antibodies specific for the *Candida* associated antigens can be used.

5. *Isolation of antigen specific immunoglobulin heavy and light chain sequences.*

In addition to providing *Candida* phosphomannan (and β-1,2-mannotriose) specific antibodies, the subject invention provides for polynucleotides encoding *Candida* specific antibodies. The polynucleotides may have a wide variety of sequences because of the degeneracy of the genetic code. A person of ordinary skill in the art may readily change a given polynucleotide sequence encoding a *Candida* specific antibody according to the present invention into a different polynucleotide encoding the same antibody. For example, the polynucleotide sequence encoding the antibody may be varied to take into account factors affecting expression such as codon frequency, RNA secondary structure, and the like.

6. *Production of recombinant human antibodies*

The antibodies of the subject invention may be produced by a variety of methods useful for the production of polypeptides, *e.g.*, *in vitro* synthesis, recombinant DNA production, and the like. Preferably, humanized antibodies are produced by recombinant DNA technology. The antigen specific antibodies of the invention may be produced using recombinant immunoglobulin expression technology. The recombinant production of immunoglobulin molecules, including humanized antibodies is described in U.S. Patent No. 4,816,397 (Boss *et al.*), U.S. Patent No. 4,816,567 (Cabilly *et al.*), U.K. patent GB 2,188,638 (Winter *et al.*), and U.K. patent GB 2,209,757 (Winter *et al.*). Techniques

for the recombinant expression of immunoglobulins, including humanized immunoglobulins, can also be found, among in Goeddel *et al.*, "Gene Expression Technology Methods" IN ENZYMOLOGY Vol. 185 Academic Press (1991), and Borreback, ANTIBODY ENGINEERING, W. H. Freeman (1992). Additional information concerning the generation, design and expression of recombinant antibodies can be found in Mayforth, DESIGNING ANTIBODIES, Academic Press, San Diego (1993).

As an example, the recombinant antibodies of the present invention may be produced by the following process:

a) constructing, by conventional molecular biology methods, an expression vector comprising a nucleotide sequence that encodes an antibody heavy chain in which the CDRs and a minimal portion of the variable region framework that are required to retain donor antibody binding specificity are derived from the human immunoglobulin, and the remainder of the antibody is derived from another human immunoglobulin, thereby producing a vector for the expression of a humanized antibody heavy chain;

b) constructing, by conventional molecular biology methods, an expression vector comprising a nucleotide sequence that encodes an antibody light chain in which the CDRs and a minimal portion of the variable region framework that are required to retain donor antibody binding specificity are derived from the human immunoglobulin, and the remainder of the antibody is derived from another human immunoglobulin, thereby producing a vector for the expression of humanized antibody light chain;

c) transferring the expression vectors to a host cell by conventional molecular biology methods to produce a transfected host cell; and

d) culturing the transfected cell by conventional cell culture techniques so as to produce recombinant antibodies.

Host cells may be cotransfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second encoding a light chain derived polypeptide. The two vectors may contain different selectable markers but, with the exception of the heavy and light chain coding sequences, are preferably identical. This procedure provides for equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and

light chain polypeptides. The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA or both.

The host cell used to express the recombinant antibody of the invention may be a bacterial cell such as *Escherichia coli,* or antigen binding fragments may be expressed in available phage display systems (see Winter *et al.* (1994) *Ann. Rev. Immunol.* 12: 433-455 and Little *et al.* (1995) *J. Biotechnol.* 41(2-3): 187-195). Preferably a eukaryotic cell or most preferably a mammalian cell, such as a Chinese hamster ovary cell, may be used. The choice of expression vector is dependent upon the choice of host cell, and may be selected by a person skilled in the art so as to have the desired expression and regulatory characteristics in the selected host cell.

The general methods for construction of the vector of the invention, transfection of cells to produce the host cell of the invention, culture of cells to produce the antibody of the invention are all conventional molecular biology methods. Likewise, once produced, the recombinant antibodies of the invention may be purified by standard procedures of the art, including cross-flow filtration, ammonium sulphate precipitation, affinity column chromatography, gel electrophoresis and the like.

7. *Preparation of diagnostic, therapeutic and prophylactic compositions*

The antibodies of the present invention may be used in conjunction with, or attached to other antibodies (or parts thereof) such as human or humanized monoclonal antibodies. These other antibodies may be reactive with other markers (epitopes) characteristic for the disease against which the antibodies of the invention are directed or may have different specificities chosen, for example, to recruit molecules or cells of the human immune system to the diseased cells. The antibodies of the invention (or parts thereof) may be administered with such antibodies (or parts thereof) as separately administered compositions or as a single composition with the two agents linked by conventional chemical or by molecular biological methods. Additionally the diagnostic and therapeutic value of the antibodies of the invention may be augmented by labeling the humanized antibodies with labels that produce a detectable signal (either *in vitro* or *in vivo*) or with a label having a therapeutic property. Some labels, *e.g.*, radionuclides may

produce a detectable signal and have a therapeutic property. Examples of radionuclide labels include ^{125}I and ^{131}I. Examples of other detectable labels include a fluorescent chromophore such as fluorescein, phycobiliprotein or tetraethyl rhodamine for fluorescence microscopy, an enzyme which produces a fluorescent or colored product for detection by fluorescence, absorbance, visible color or agglutination, which produces an electron dense product for demonstration by electron microscopy; or an electron dense molecule such as ferritin, peroxidase or gold beads for direct or indirect electron microscopic visualization. Labels having therapeutic properties include drugs for the treatment of candidiasis such as are described below.

The subject invention also provides for a variety of methods for treating and/or detecting *Candida* cells. These methods involve the administration to a patient of *Candida* specific antibodies, either labeled or unlabeled. One method of detecting *Candida* cells in a human involves the step of administering a labeled *Candida* specific antibody (labeled with a detectable label) to a human and subsequently detecting bound labeled antibody by the presence of the label. Alternatively, the *Candida* specific antibodies may be linked or conjugated to a therapeutic molecule such as ricin or other toxins.

The recombinant antibodies of this invention may also be used for the selection and/or isolation of human monoclonal antibodies, and the design and synthesis of peptide or non-peptide compounds (mimetics) which would be useful for the same diagnostic and therapeutic applications as the antibodies (*e.g.*, Saragovi *et al.*, 1991 *Science* 253: 792-795).

When the *Candida* specific antibodies of the invention are used *in vivo*, the antibodies are typically administered in a composition comprising a pharmaceutical carrier. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for delivery of the monoclonal antibodies to the patient. Sterile water, alcohol, fats, waxes, and inert solids may be included in the carrier. Pharmaceutically accepted buffering agents or dispersing agents may also be incorporated into the pharmaceutical composition.

The antibody compositions of the invention may be administered to a patient in a variety of ways. Preferably, the compositions may be administered parenterally, *i.e.*, subcutaneously, intramuscularly or intravenously. Aerosol formulations are also expressly contemplated. Injectable forms of administration are sometimes preferred for maximal systemic effect against systemic infections and infections of the respiratory tract and the deep tissues. When long term administration by injection is necessary, medi-ports, in-dwelling catheters or automatic pumping mechanisms may be used. Thus, this invention provides compositions for parenteral administration which comprise a solution of the human antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, *e.g.*, water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well-known sterilization techniques.

The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, *etc.* The concentration of antibody in these formulations can vary widely, *e.g.*, from less than about 0.5%, or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, *etc.*, in accordance with the particular mode of administration selected.

A preferred dose of antibody for systemic administration of the antibodies of the present invention are in the range of about 0.1 to about 5 mg/kg of body weight. A more preferred dose is in the range of about 0.5 to about 2.0 mg/kg, most preferably about 1.0 to about 1.5 mg/kg. Human or other mammalian subjects are treated with multiple doses of antibody pharmaceuticals on an appropriate schedule, for example, a schedule that results in and maintains substantially saturating antibody levels or significant opsonization levels in the blood or infected tissue of a patient undergoing treatment according to the methods of the present invention. For example, a one-time dose of a chimeric antibody may be administered as described in Clark *et al.*, " Effect of a chimeric

antibody to tumor necrosis factor-(alpha) on cytokine and physiologic responses in patients with severe sepsis - A randomized, clinical trial" in *Crit. Care Med.* 26:1650-59 (1998).

Local or mucocutaneous infections would be treated by topical application of the therapeutic antibody compositions of the present invention. For oral delivery, for example, the pharmaceutical compositions may be administered in the form of a cream or a wash that can be applied by, *e.g.*, swab or by rinsing at period intervals. These compositions also may be formulated into buccal suppositories for release, *e.g.*, from the oral region over an extended period of time. In an alternative embodiment, tablets or oral insert or gum may be utilized as delivery vehicles. For vaginal delivery, the composition may be administered in a cream formulation, vaginal suppository or insert, as is well known in the art.

Pharmaceutically effective amounts would be those amounts of the proposed pharmaceutical compositions required to yield a positive effect. Positive effects include a reduction of organism load in the subject, death or inactivation of the organism, or complete or nearly complete elimination of the infecting organism from the body. Preferably, the patient has an infection as measured by any appropriate testing parameter, which is reduced at least 100-fold, more preferably 1,000-fold, and even more preferably is undetectable after treatment.

Yet other embodiments of the invention are directed to compositions of the invention which can be used in combination with other agents (*e.g.*, anti-fungal agents) to maximize the effect of the compositions in an additive or synergistic manner. Agents that may be effective in combination with the compositions of the invention include other drugs and treatments which are known or suspected to have a positive effect against a *Candida* organism. Such agents include, but are not limited to, flucytosine, mycoconazole, fluconazole, itraconazole, ketoconazole, griseofulvin, amphotericin B and derivatives, modifications and combinations of these agents. Other agents are described, for example, in U.S. Patent No. 5,679,648 to McCaffrey *et al.* (1977).

Actual methods for preparing parenterally administrable compositions and adjustments necessary for administration to subjects will be known or apparent to those

skilled in the art and are described in more detail in, for example, REMINGTON'S PHARMACEUTICAL SCIENCE, 15th Ed., Mack Publishing Company, Easton, Pa. (1980), which is incorporated herein by reference.

8. *Diagnostic kits for detecting diseased tissues and Candida cells*

A kit can be prepared that comprises an antibody according to the present invention capable of binding to a diseased tissue or to *Candida*. These kits can be used in conjunction with existing histological staining techniques to determine more quickly, as well as more accurately, what disease is present and the extent of infection or stage of disease. This would be useful for purposes of diagnosing, detecting and/or determining what therapy or therapies may be appropriate in treating a particular subject's disease.

The preferred kit would have the antibody prepared for contact with a tissue or biological fluid sample, for example. The sample then would be incubated with the antibody, as would be known for conventional methods used in the art. After incubation with kit antibody, the cells and/or tissue would be examined for the presence or absence of binding. Standard assays to be used in such kits include, but are not limited to latex agglutination, radio immunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) or other suitable antigen detection system.

In light of the foregoing general discussion, the specific examples presented below are illustrative only and are not intended to limit the scope of the invention. Other generic configurations will be apparent to one skilled in the art.

EXAMPLES

***Example 1*: Materials and Methods.**

The organisms and culture conditions for preparing the cell line which produces the C3.1 antibody is as follows. *C. albicans CA-1* was started from frozen glycerol stocks as previously described (Han *et al.*, 1995 *Infect. Immun.* 63: 2714-9; Han *et al.*, 1998 *Infect. Immun.* 66: 5771-5776; and Kanbe *et al.*, 1993 *Infect. Immun.* 61: 2578-2584) and was grown as hydrophilic stationary-phase yeast cells in glucose-yeast extract-

peptone (GYEP) broth at 37°C. Hydrophilic yeast cells were washed, suspended to the desired yeast cell concentration in Dulbecco's phosphate-buffered saline (DPBS, Sigma Chemical Co., St. Louis, MO.), and used to infect mice.

The BALB/c female mice obtained from Charles River Laboratories (Kingston, N.Y.), were 6 to 8 weeks old and were used throughout this study.

The MAb C3.1 was one of several MAbs, including the protective MAb B6.1, that were isolated through hybridoma techniques from the L-mann-vaccinated mice (Han *et al.*, 1995). MAb C3.1 was produced in serum-free medium and ammonium sulfate precipitated by LigoCyte Pharmaceuticals, Inc. (Bozeman, MT). MAb B6.1 served as a positive control antibody and was characterized as described previously (Han *et al.*, 1997 *J. Infect. Dis.* 175: 1169-1175; Han *et al.*, 1998). MAb B6.1 was produced in serum-free medium and ammonium sulfate precipitated by LigoCyte Pharmaceuticals, Inc. (Bozeman, MT). Antibody titers were measured by agglutination with either whole *C. albicans* yeast cells or mannan coated latex beads (see Han *et al.*, 1995; and Han *et al.*, 1998).

Isotyping of the MAb C3.1 was performed as follows. The MAb C3.1 isotype was detected by capture enzyme-linked immunosorbent assay (ELISA), and was confirmed by immuno-double diffusion (Ouchterlony) techniques. All anti-mouse immunoglobulins were purchased from Sigma.

Mannan extraction and acid hydrolysis. *C. albicans* yeast cells, grown as described above, were treated with β-mercaptoethanol to yield a mannan extract, which was further fractionated on a concanavalin A affinity column as before (Kanbe *et al.*, 1993 *Infect. Immun.* 61: 2578-2584). The extract was hydrolyzed by boiling in 10 mM HCl, neutralized, and applied onto a P-2 (Bio-Rad, Richmond, CA) column as described (Han *et al.*, 1997 *Infect. Immun.* 4100-4107). The various fractions eluted from the P-2 column were tested for their ability to react with MAb C3.1 in the same manner as previously described for identification of the epitope specificity of MAb B6.1 (*Id.*).

Fluorescence Microscopy. Distribution of the C3.1 epitope on yeast cells was determined by an indirect immunofluorescence (IFA). MAb C3.1 was reacted with yeast cells, washed, and interacted with fluorescein-labeled anti-mouse IgG or anti-mouse IgM. The cells were observed by fluorescence and by phase-contrast microscopy.

Example 2: **MAb C3.1 Transfers Protection Against Disseminated Candidiasis.**

Protection against *Candida* infections by passive transfer. MAb C3.1 was immediately used or stored at -20°C, treated at 56°C for 30 min before use, or absorbed with C. *albicans* yeast cells (Han *et al.*, 1995; Han *et al.*, 1998; Han *et al.*, 1998 *J. Infect. Dis. (in press)*). The prophylactic effect of MAb C3.1 was tested against experimental disseminated candidiasis and *Candida* vaginal infection as follows:

1) Against disseminated candidiasis:

0 h, Treat with MAb C3.1, intra peritoneally (i.p.)

4 h, Challenge with *C. albicans* ($5x10^5$ yeast cells), intravenously (i.v.)

48 h, Determine kidney colony forming units (CFU) or continue to measure survival times.

2) Against vaginal infection:

0 h, Inject estradiol (0.5 mg/mouse), subcutaneously (s.c.)

72 h, Treat with MAb C3.1 (0.5 ml/mouse), i.p. or i.vg. (For i.vg., 100 µl of Mab C3.1 was given).

76 h, Challenge with *C. albicans* ($5x10^5$ yeast cells), i.vg.

120 h, Determine vaginal CFU (Han *et al.*, 1998 *Infect Immun* 66:5771-5776).

Statistical significance of differences in survival times was calculated by use of the Kaplan-Meier test (Systat 7.0, SPSS Inc. Chicago, IL). For other analyses, the Student's t-test was used. *P*-values were considered statistically significant if they were less than 0.05.

Figure 1 demonstrates that MAb C3.1 has a prophylactic effect against *Candida*. In Panel (A), BALB/c mice were given unheated MAb C3.1 (0-1), MAb heated at 56°C for 30 min (H-C3.1), *C. albicans*-absorbed MAb C3.1 (A-C3.1) or DPBS (buffer diluent) i.p.

In Panel B of Figure 1, mice were given MAb C3.1, MAb B6.1 or DPBS as a control. In both panels, the animals were challenged i.v with $5x10^5$ viable yeast cells and susceptibility to disseminated disease was assessed by determining kidney CFU (A) and by survival curves (B).

Mice that received the unheated or heated MAb C3.1 (Figure 1A) had 86% and 88% fewer CFU, respectively, than mice that received DPBS ($P < 0.001$). Mice given the absorbed serum developed almost the same number of CFU as control mice that received the DPBS. Bars show standard errors. Mice that received MAb C3.1 had survival times similar to animals given MAb B6.1. Their mean survival times were significantly longer than animals given DPBS ($P<0.05$). The conclusion is that MAb C3.1 enhances resistance of mice against disseminated candidiasis.

***Example 3*: <u>MAb C3.1 Has Prophylactic Effect against *Candida* Vaginal Infection</u>.**

Pseudoestrus mice were given MAb C3.1 intra peritoneally (i.p.) (Figure 2A) or intra vaginally (i.vg.) (Figure 2B) before an i.vg. challenge with yeast cells ($5x10^5$). Vaginal CFUs were compared with CFU from animals that were given unheated MAb C3.1 (C3.1), heat treated at 56°C for 30 min (H-C3.1), *C. albicans*-absorbed C3.1 (A-0.1), or DPBS (diluent) as described in Figure 2A.

In Figure 2A, mice that received the unheated (C3.1) or heated (H-C3.1) developed 60% and 49% fewer CFU, respectively, than DPBS-control mice. Mice that received the absorbed C3.1 (A-C3.1) or DPBS developed similar CFU.

In Figure 2B, mice that received the unheated or heated MAb C3.1 developed approximately 86% fewer CFU than DPBS control mice. This CFU reduction is similar to that observed due to administration of MAb B6.1. Mice given the *C. albicans*-absorbed C3.1 resulted in similar CFU as the DPBS-control mice.

In both panels, significant differences were found between mice that received either the unheated MAb C3.1 or heated MAb C3.1 and DPBS controls ($P < 0.05$). Bars show standard errors.

Example 4: Antibodies of the IgG Class that Are Protective Against *C. Tropicalis*

The techniques described in Example 1 are followed using yeast cells of the *C. tropicalis* species to prepare an L-mann immunogen. BALB/c female mice obtained from Charles River Laboratories (Kingston, N.Y.), that are 6 to 8 weeks old are immunized. Hybridomas are produced and screened through hybridoma techniques from the L-mann-vaccinated mice per the procedures of Han *et al.*, 1995. Antibody titers are measured by agglutination with either whole *C. tropicalis* yeast cells or mannan coated latex beads by the techniques of Han *et al.*, 1995; and Han *et al.*, 1998. Selected antibodies to be isotyped are detected by capture enzyme-linked immunosorbent assay (ELISA), and isotype is confirmed by immuno-double diffusion (Ouchterlony) techniques. Protection against *Candida* infections by passive transfer is determined by the technique shown in Example 3.

Example 5: Additional IgG Antibodies Against *Candida albicans*

The techniques described in Example 1 are followed using yeast cells of the *C. albicans* species to prepare an L-mann immunogen. BALB/c female mice obtained from Charles River Laboratories (Kingston, N.Y.), that are 6 to 8 weeks old are immunized. Hybridomas are produced and screened through hybridoma techniques from the L-mann-vaccinated mice per the procedures of Han *et al.*, 1995. Antibody titers are measured by agglutination with either whole *C. albicans* yeast cells or mannan coated latex beads by the techniques of Han *et al.*, 1995; and Han *et al.*, 1998. Selected antibodies to be isotyped are detected by capture enzyme-linked immunosorbent assay (ELISA), and isotype is confirmed by immuno-double diffusion (Ouchterlony) techniques. Protection against *Candida* infections by passive transfer is determined by the technique shown in Example 3.

Example 6: Treatment of *Candida* Infection in Human Patients

For the treatment of disseminated disease, patients who develop evidence of disseminated disease should receive the antibody i.v. or i.p. or i.m., alone or in combination with other antifungal agents. For the prevention of candidiasis, high-risk patients should be identified (*e.g.*, those who will undergo abdominal surgery, open heart surgery, kidney transplants, bone marrow transplants, receive indwelling catheters, corticosteriods, broad spectrum antibiotics), and the antibody pharmaceutical compositions described above should be administered i.v. or i.m. prior to the procedure. For the treatment of vaginal *Candida* infections, the antibody pharmaceutical compositions described above are administered intravaginally, as well as i.v. or i.p. or i.m., alone or in combination with other antifungal agents.

Example 7: Use of Test Kits to Detect *Candida* Infection

The antibodies as described above are used in a capture antigen format to capture *Candida* antigen in the serum or vaginal secretions from an infected patient. Such kits are further prepared with agents to detect the binding of the antibody to such antigens.

It should be understood that the foregoing discussion and examples present merely present a detailed description of certain preferred embodiments. It therefore should be apparent to those of ordinary skill in the art that various modifications and equivalents can be made without departing from the spirit and scope of the invention. All articles, patents and patent applications that are identified above are incorporated by reference in their entirety.

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2. **Han, Y. and J.E. Cutler,** 1995. Antibody response that protects against disseminated candidiasis. *Infect.Immun.* 63:2714-2719.

3. **Han, Y. and J.E. Cutler**, 1997. Assessment of a mouse model of neutropenia and the effect of an anti-candidiasis monoclonal antibody in these animals. *J.Infect.Dis.* 175:1169-1175.

4. **Han, Y., T. Kanbe, R. Cherniak and J.E. Cutler**, 1997. Biochemical characterization of *Candida albicans* epitopes that can elicit protective and nonprotective antibodies. *Infect.Immun.* 65:4100-4107.

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10. **Yuan, R., A. Casadevall, G. Spira, and M.D. Scharff**, 1995. Isotype switching from IgG3 to IgG1 converts a nonprotective murine antibody to *Cryptococcus neoformans* into a protective antibody. *J.Immunol.* 154:1810-1816.

11. **Yuan, R.R., G. Spira, J. Oh, M. Paizi, A. Casadevall and M.D. Scharff**, 1998. Isotope switching increases efficacy of antibody protection against *Cryptococcus neoformans* infection in mice. *Infect.Immun.* 66:1057-1062.

We claim:

1. An isolated IgG antibody that specifically binds to a carbohydrate antigen of the cell wall of a yeast from the *Candida* genus, said antibody being protective against infection of a mammalian host by the yeast.

2. The antibody of claim 1, wherein the antibody specifically binds to an acid-labile component of the phosphomannan complex of the cell wall of the yeast.

3. The antibody of claim 2, wherein the acid-labile component to which the antibody specifically binds is a β-1,2-linked oligomannosyl residue.

4. The antibody of claim 2, wherein the acid-labile component to which the antibody specifically binds is a β-1,2-mannotriose residue.

5. The antibody of any of claims 1 to 4, wherein the yeast is selected from the group consisting of *C. albicans, C. glabrata* and *C. tropicalis* and strains thereof.

6. The antibody of claim 5, wherein the yeast is *C. albicans*.

7. The antibody of any of claims 1 to 4, wherein said antibody is protective against a type of candidiasis selected from the group consisting of disseminated candidiasis and mucocutaneous candidiasis.

8. The antibody of any of claims 1 to 4, wherein the antibody is of an isotype selected from the group consisting of IgG1, IgG2 and IgG3.

9. The antibody of claim 8, wherein the antibody is of the IgG3 isotype.

10. The antibody of claim 9, wherein the antibody is antibody C3.1.

11. The isolated antibody of claim 5, wherein the antibody is selected from the group consisting of a human antibody, a chimeric antibody or a humanized antibody.

12. A pharmaceutical composition comprising the isolated antibody of claim 5 formulated with pharmaceutically acceptable carriers and excipients.

13. The pharmaceutical composition of claim 12, wherein the composition is formulated for topical administration.

14. The pharmaceutical composition of claim 12, wherein the composition is formulated for systemic administration.

15. The pharmaceutical composition of claim 12, wherein the composition is formulated as an aerosol.

16. The pharmaceutical composition of claim 12, wherein the composition is formulated in a therapeutically effective unit dose form.

17. The pharmaceutical composition of claim 12, further comprising one or more other therapeutic agents.

18. The pharmaceutical composition of claim 17, wherein said one or more other therapeutic agents is an antifungal agent.

19. The pharmaceutical composition of claim 17, wherein said one or more other therapeutic agents is an antibody.

20. A method of treating or preventing candidiasis in a mammalian subject, comprising the step of administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim 12.

21. The method of claim 20, wherein the candidiasis is disseminated candidiasis.

22. The method of claim 20, wherein the candidiasis is mucocutaneous candidiasis.

23. A method of treating candidiasis in a subject, comprising the step of administering to a subject a therapeutically effective amount of an antibody according to claim 5.

24. A method of inhibiting or preventing onset of candidiasis in a mammalian subject comprising the step of administering to a subject in need thereof a protective amount of an IgG antibody, wherein the antibody specifically binds to a carbohydrate antigen of the cell wall of a yeast from the *Candida* genus.

25. The method of claim 24, wherein the yeast is selected from the group consisting of *C. albicans, C. glabrata* and *C. tropicalis* and strains thereof.

26. The method of claim 24, further comprising the step of administering a protective amount of an anti-*Candida* IgM antibody.

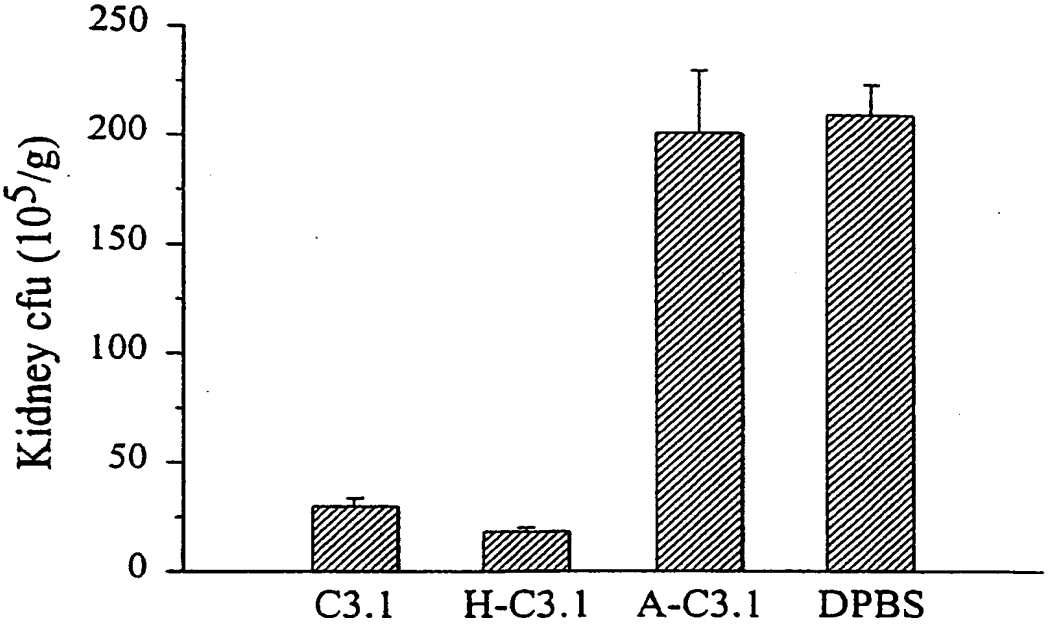
27. The method of claim 26, wherein the IgM antibody and IgG antibody are administered at about the same time.

28. The method of claim 26, wherein the IgM antibody and IgG antibody are administered at different times.

29. A diagnostic kit comprising the antibody of any of claims 1 to 4 together with a reagent for detecting binding of the antibody to a carbohydrate antigen of the cell wall of a yeast from the *Candida* genus.

30. A hybridoma cell that expresses the antibody of any of claims 1 to 4.

FIG. 1A


250
200
150
100
50
0
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C3.1
H-C3.1
A-C3.1
DPBS

FIG. 1B

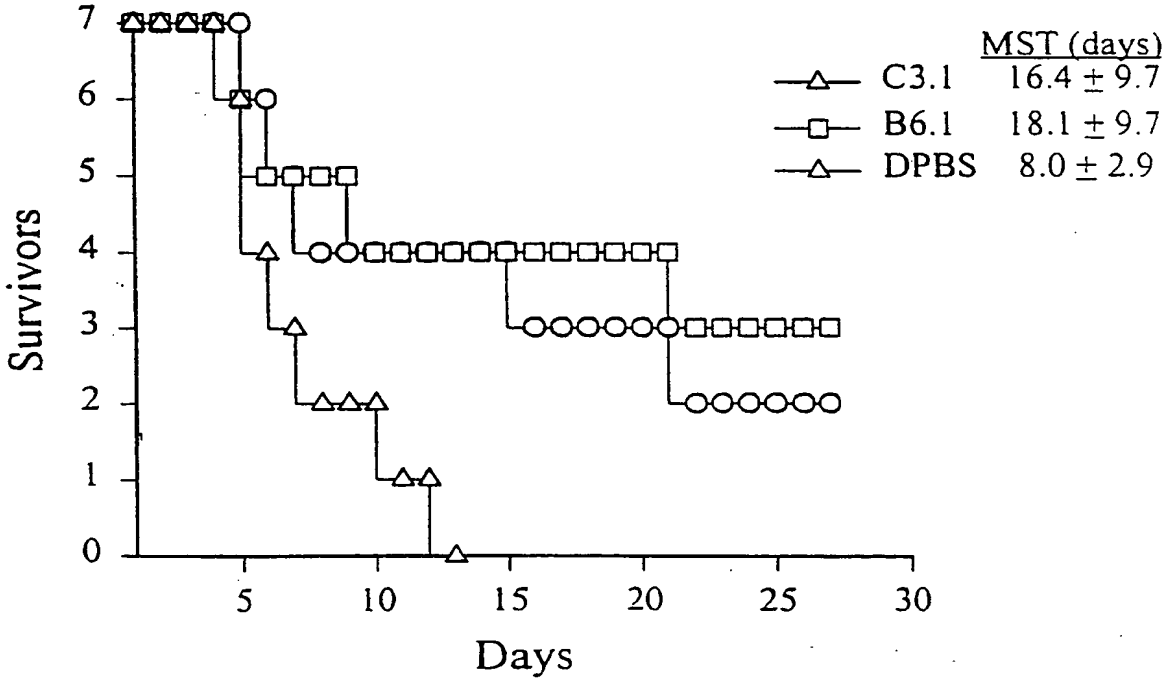
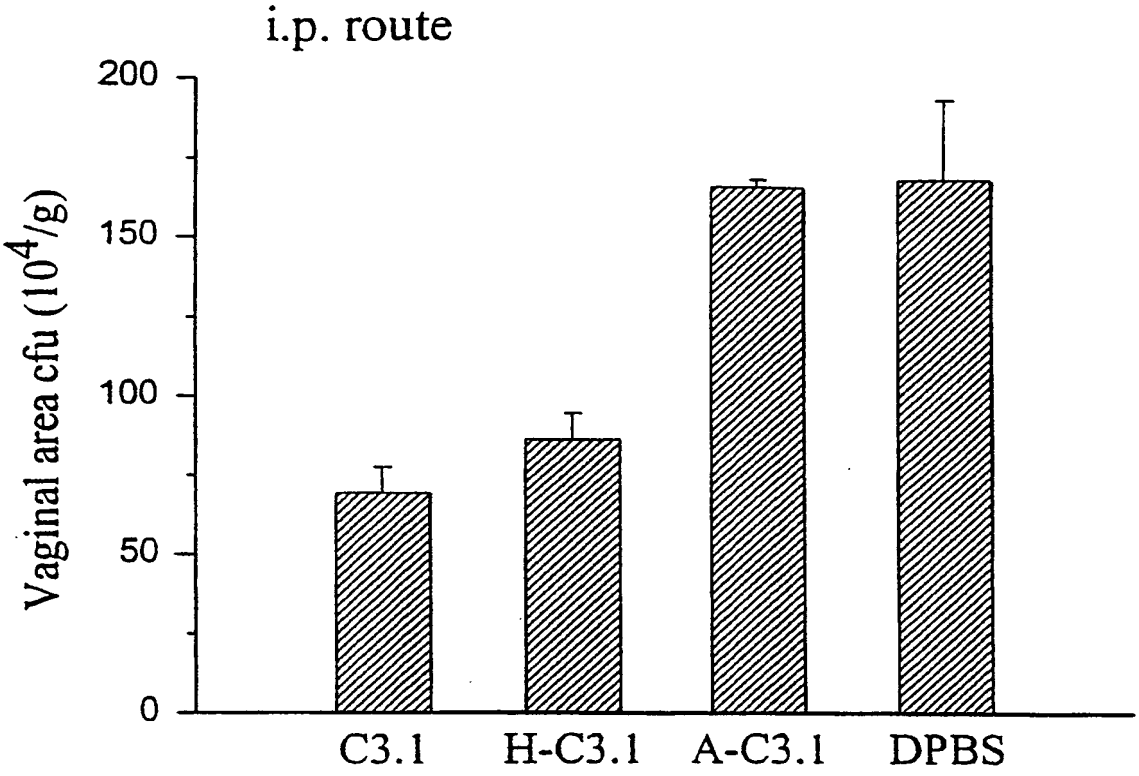

7
6
5
4
3
2
1
0
Survivors
5
10
15
20
25
30
Days
MST (days)
C3.1 16.4 ± 9.7
B6.1 18.1 ± 9.7
DPBS 8.0 ± 2.9

FIG. 2A


i.p. route
Vaginal area cfu (10^4/g)
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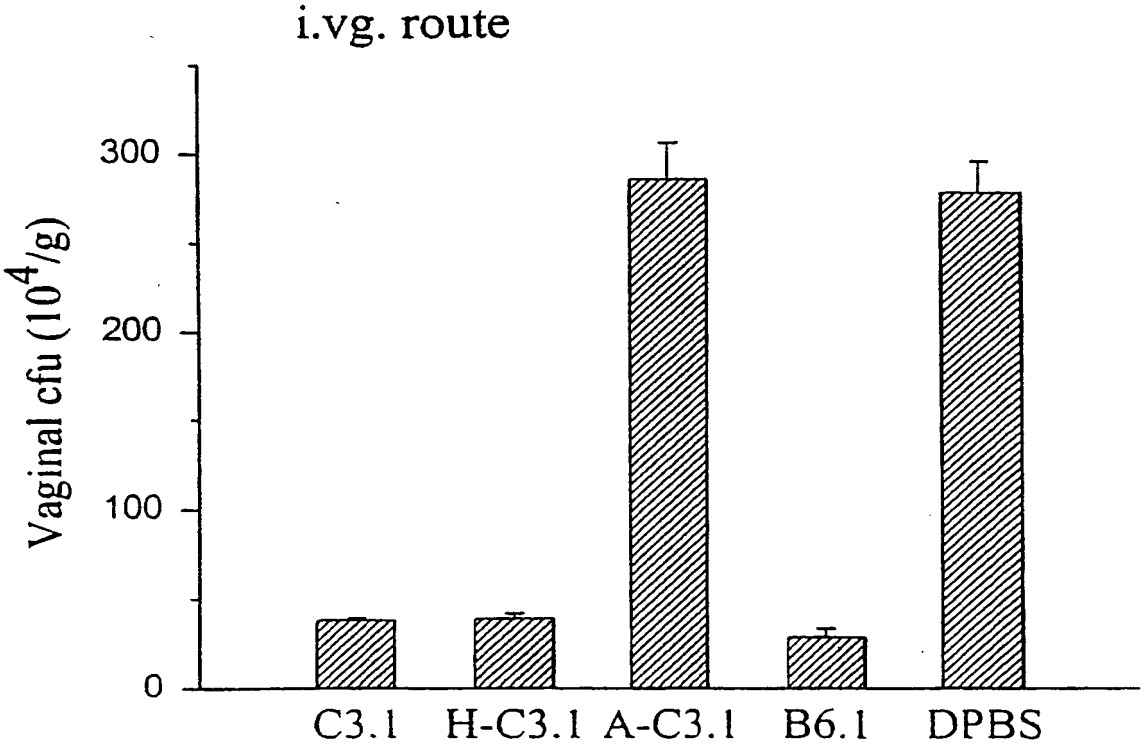
150
100
50
0
C3.1
H-C3.1
A-C3.1
DPBS

FIG. 2B


i.vg. route
Vaginal cfu (10^4/g)
300
200
100
0
C3.1
H-C3.1
A-C3.1
B6.1
DPBS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/05279

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07K 16/00; A61K 39/395

US CL : 530/387.1, 387.5, 388.1, 388.5; 424/130.1, 137.1, 141.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/387.1, 387.5, 388.1, 388.5; 424/130.1, 137.1, 141.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, STN, MEDLINE, BIOSIS, Candida albicans, antibody, IgG, IgM, acid-labie, oligomannosyl.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — A	KAMEL, S.M. et al. Production and Characterization of Murine Monoclonal Antibodies to Histoplasma capsulatum Yeast Cell Antigens. Infection And Immunity. March 1989, Vol. 57, No. 3, pages 896-901, especially Abstract.	1-9, 11-30 ------ 10
X	TOJO, M. et al. Preparation of Monoclonal Antibodies Reactive with β-1,2-linked Oligomannosyl Residues in the Phosphomannan-Protein Complex of Candida albicans NIH B-792 Strain. Clin. Chem. 1988, Vol. 34, No. 3, pages 539-543, especially page 539.	1-9, 11-30

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 15 JUNE 2000	Date of mailing of the international search report 05 JUL 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LI LEE Telephone No. (703) 308-0196 JOYCE BRIDGERS PARALEGAL SPECIALIST CHEMICAL MATRIX

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/05279

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KANBE, T. et al. Evidence for Adhesin Activity in the Acid-Stable Moiety of the Phosphomannoprotein Cell Wall Complex of Candida albicans. Infection And Immunity. May 1994, Vol. 62, No. 5, pages 1662-1668, especially Abstract.	1-9, 11-30
X	TAVARES, D. et al. Immunoprotection against systemic candidiasis in mice. International Immunology. 1995, Vol. 7, No. 5, pages 785-796, especially Abstract.	1-9, 11-30